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## 10. Resource recovery and reduction of oily hazardous wastes via biosurfactant washing and bioremediation

Helen E. Connolly<sup>1</sup>, Pattanathu K.S.M. Rahman<sup>1</sup>, Ibrahim M. Banat<sup>2</sup>  
and Richard A. Lord<sup>1</sup>

<sup>1</sup>*School of Science and Engineering, Teesside University, Middlesbrough – TS1 3 BA, UK*

<sup>2</sup>*School of Biomedical Science, University of Ulster, Coleraine – BT52 1SA, Northern Ireland, UK*

**Abstract.** Physico-chemical washing of oil-contaminated soils with biosurfactant offers a novel pre-treatment method which could potentially enhance subsequent bioremediation. Although literature reviews and our pilot studies using artificially oil “spiked” soils under a similar washing regime had indicated that oil would be released during soil-washing, it was soon apparent that this was not the case for weathered, oil-contaminated waste soils where virtually no oil released into solution occurred. Furthermore, we frequently detected an apparent increase in soil hydrocarbon contamination levels in analysis after washing. This was demonstrated to be partly an artefact of the smaller grain size fraction used for the standard analytical protocol (<2 mm), compared to that used in the standard washing protocol (<4 mm). The apparent increased contamination in the former resulted from the efficient transfer of oil contamination from the coarser particles (i.e. 2-4 mm) to the clay component during soil-washing. We concluded that the envisaged combination of biosurfactant and low intensity soil-washing was unlikely to remove oil from soils or other oily hazardous wastes due to the potent transfer of contaminants to the fine-grain fraction which is inherent in most conventional soil-washing processes. Biosurfactants however can potentially offer technically and economically competitive alternatives to chemical surfactants derived from fossil fuels.

Correspondence/Reprint request: Dr. Helen E. Connolly, School of Science and Engineering, Teesside University Middlesbrough – TS1 3 BA, UK. E-mail: H.Connolly@tees.ac.uk

## 1. Introduction

Prior to July 2004, when a ban on co-disposal with domestic refuse was introduced under the 'Landfill Directive', contaminated soils accounted for about 60% of hazardous waste arising in the UK. Sustainable alternatives to "excavate and remove" (to landfill) to deal with contaminated soils utilise physical, chemical or biological processing which allow reuse of the treated material on site. Although various process-based remediation technologies are now available (e.g. soil-washing, thermal desorption, chemical treatments, bioremediation [1,2]), these are not readily applicable to smaller urban Brownfield sites due to the large area involved, capital equipment and timescales required. We investigated using the combination of two existing processes (soil-washing and bioremediation) with the use of natural soaps (biosurfactants) under laboratory conditions with the aim of developing novel means of recycling oily soils and similar waste products and develop a process suitable for use a treatment centre.

Soil-washing involves mixing soil and water as a slurry and then separating the particles into grain size fractions [3,4,5]. During this process any fine-grained metal-rich contamination and oils become associated with the clay fraction, whereas the sand and gravel fractions are left clean and may be reused as aggregate, reducing the final volume requiring disposal as hazardous waste. Soil-washing is already widely used in Europe and in recent years has been successfully applied in the UK to remediate contaminated sites and to establish soil treatment centres. The availability of conventional on-site soil-washing from UK remediation companies increased five-fold during the past five years, although regulatory issues surrounding the reuse of fractions derived from waste soil [6] are currently a limiting factor to widespread implementation of this potentially sustainable and low-carbon remediation method in fixed treatment centres.

Bioremediation of soils involves mechanical aeration, which allows naturally occurring soil organisms (bacteria) to breakdown hydrocarbon contaminants to less harmful by-products in a manner akin to composting, eventually producing carbon dioxide and water. It is usually achieved by turning soils in elongated piles (windrows) or by stacking above an aeration system (biopiles). Liquids or slurries can be aerated in a tank (bioreactor), which is advantageous in controlling temperature and other parameters.

Biosurfactants are surface-active compounds produced by micro-organisms (e.g. bacteria, fungi, actinomycetes) to enable them to digest hydrocarbons which are otherwise immiscible in water [7]. They are biodegradable non-toxic and eco-friendly materials. Chemically they are combinations of fat, carbohydrate or protein, such as glycolipid, phospholipid, lipopeptide, sophorolipids [8]. The chemical nature of the product varies with the organism: For example rhamnolipid is produced by *Pseudomonas aeruginosa*, sophorolipid by *Rhodococcus erythropolis*. Rhamnolipid is an extensively studied biosurfactant which is now commercially available for industrial applications [9]. There are reports of the use of biosurfactants for bioremediation of soil samples spiked with petroleum hydrocarbons [8,10,11,12,13], but not of actual samples from gas work sites, diesel contaminated soil or oily mill scale.

Soil-washing trials with petroleum-based surfactants have shown that their addition enhances the removal of non-aqueous phase liquids (NAPLS) and can promote biodegradation [14]. It was anticipated that the proposed use of biosurfactants during soil-washing in this study would ensure that any surfactant residues in washed solids were biodegradable, and, after separation of useful oil, that the liquid eluates were likewise more amenable to bioremediation [15]. This could optimize any subsequent composting by reducing contaminant toxicity, or even eliminate the need to treat the bulk

of the solids. The reduced volume of contaminated liquid produced could be treated intensively in controlled conditions in a bioreactor.

An additional aim of this study was to apply biosurfactant-assisted soil-washing technologies to the reduction, reuse and recycling of granular oily wastes other than soils. For example, iron ore feed stocks might be recovered from treatment of oily mill scale, a secondary product that is currently land filled. Following introduction of the European Landfill Directive in the UK this approach might also be used to reduce the hazardous properties of wastes destined for disposal and to fully meet the Waste Acceptance Criteria for organic contaminants.

Our experimental approach was to select 10 wastes and to sample these onsite for laboratory-scale experiments to determine the following:

- optimized washing conditions, based initially on British Standard 12457-3 [16], but with analysis of solid residues and eluates
- the feasibility of separating oil during biosurfactant recovery for energy or reuse
- the effect on the speed or extent of secondary bioremediation treatment when biosurfactants are used
- the advantages of bioremediating the washed solids and the oily liquids separately
- the possibility of in-cycle production of biosurfactant during washing

The primary objective was to evaluate the use of biosurfactant soil-washing as a process-based treatment of particulate wastes, as a precursor to oil recovery or enhanced bioremediation, and as a resource recovery or waste minimisation. The characterisation of commercial biosurfactants and development and testing of a soil washing methodology and are reported in this manuscript. The laboratory method used was based on a two-stage batch leaching test developed for the characterisation and compliance testing of hazardous waste destined for disposal. As such it is of much lower intensity than conventional soil-washing tests but is designed to mimic long term equilibration between waste and groundwater.

## **2. Experimental methods and results**

### **2.1. Waste identification & sampling**

Seventeen potential target waste streams were identified as potentially treatable by soil washing technology. This list was consulted, along with the European Waste Catalogue, when sourcing potential waste streams for the project. The ideal waste would have the following characteristics; granular, oily, potentially hazardous and would have a re-use value if cleaned. Examples of potential waste streams are highlighted in Table 1.

### **2.2. Waste characterization and analytical development**

Three waste samples: gas works soil, oily mill scale and garden soil (uncontaminated control), were used in soil washing trials, and consequently a full suite of chemical analysis were carried out by a commercial laboratory (Severn Trent Laboratories) to determine the full extent of contamination in the samples, including total petroleum hydrocarbon (TPH), metal and polycyclic aromatic hydrocarbon (PAH) content.

As total organic carbon (TOC) would be affected by content of coal, peat or carbonate rock fragments, washing success would be determined by removal of TPH as an indicator of oil content and the incumbent hazardous properties. The waste samples

**Table 1.** Waste sources identified as potential material for soil washing trials with biosurfactants.

Unwashed gas works soil
Filter cake from soil washing at a former gas works site
Oily mill scale*†
Fresh diesel spill contaminated soil
Historically hydrocarbon contaminated soil
Metalworking swarf grindings*
Oily rags/personal protective equipment
Tank scale
Diesel contaminated soil from underground storage tank leak
Oily tank crust
Oily used polypropylene sorbents*
Oilfield drill cuttings
Tertiary-butyl-tin contaminated sediments
Hydraulic fluid contaminated sediments
Rail ballast*
Phenolic moulding sand
Municipal road sweepings
Smelter Zn-Cd contaminated soil
Refinery tank sludge
* indicates a high reuse potential if decontaminated
† typically non-hazardous but oily

**Table 2.** Full suite chemical analysis data for the wastes: gasworks soil, oily mill scale and garden soil.

Analyte (ppm unless stated)	Waste Tested		
	Gasworks soil	Oily mill scale	Garden soil
Arsenic	66	20	17
Boron	0.91	1.5	4.7
Cadmium	<0.50	<0.50	0.77
Hexavalent Chromium	<0.10	1.6	<0.10
Chromium	28	150	31
Copper	29	170	65
Lead	340	180	340

**Table 2.** Continued

Mercury	0.9	<0.20	0.25
Nickel	26	180	33
Selenium	1.7	<0.30	0.81
Zinc	140	59	370
Cyanide (Free)	<5.0	<5.0	<5.0
Cyanide (Total)	140	<2.0	8.5
Phenol	2.6	<0.50	0.86
Sulphate (%)	0.36	<0.020	0.1
Sulphide	<5.0	<5.0	15
Sulphur (Total %)	0.8	0.093	0.22
Thiocyanate	0.62	0.64	0.7
<b>TOC (%)</b>	<b>4.3</b>	<b>6.1</b>	<b>7.2</b>
pH	8.8	9.5	7.7
TPH (>C6-C10)	<50	580	<50
TPH (>C10 - C20)	430	2000	140
TPH (>C20-C40)	1000	29000	380
<b>TPH Total (&gt;C6 - C40)</b>	<b>1400</b>	<b>31000</b>	<b>520</b>
naphthalene	3	<0.50	3.8
acenaphthylene	<0.50	<0.50	<0.50
acenaphthene	0.97	<0.50	2.3
fluorene	1.1	<0.50	1.6
phenanthrene	6.9	<0.50	14
anthracene	3.3	<0.50	5.2
fluoranthene	16	<0.50	20
pyrene	11	<0.50	14
benzo(a)anthracene	5.3	<0.50	7.7
chrysene	4.8	<0.50	8.2
benzo(b)fluoranthene	5.5	<0.50	5.1
benzo(k)fluoranthene	3.3	<0.50	3.6
benzo(a)pyrene	8.1	<0.50	6.6
dibenzo(ah)anthracene	0.55	<0.50	0.66
benzo(ghi)perylene	5.9	<0.50	6.9
indeno(123cd)pyrene	6.2	<0.50	5.3
<b>PAH (Total)</b>	<b>81</b>	<b>&lt;2.0</b>	<b>110</b>

from the washing tests were also analysed for TPH content by the same commercial laboratory (Severn Trent Laboratories).

Chemical analysis was performed in triplicate on sub-samples of all wastes to evaluate the heterogeneity of chemical content of the bulk samples after mixing. Data indicated that the TPH content of three replicate (10g) samples of soil, removed from a 20kg homogenised sample, were significantly similar to suggest thorough mixing had taken place.

All chemical analyses for the three samples are listed in Table 2. Due to the presence of total petroleum hydrocarbons (TPH) fractions, the soils could potentially be categorized as hazardous by the European Waste Catalogue definitions. The oily mill scale could also fail Waste Acceptance Criteria if presented for landfill based on their Total Organic Carbon (TOC) levels above 6 % (unless LOI is <10 %). The OMS has a high TPH content comprising mostly longer chain hydrocarbons (C20 to 40), but no PAH.

## **2.3. Biosurfactants characterization and behaviour**

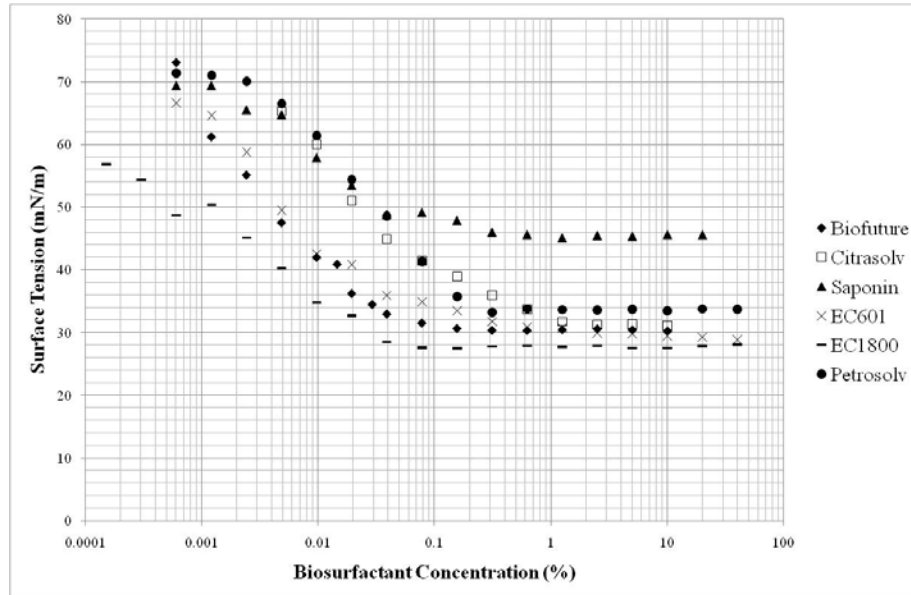
### **2.3.1. Determination of biosurfactant critical micelle concentration**

It has been postulated that pollutant removal from soil during washing trials may be dependant on biosurfactant characteristics and the formation of micelle structures by biosurfactant monomers in solution, at a concentration above the critical micelle concentration or CMC. The critical micelle concentration is the lowest concentration of surfactant that produces the minimum achievable surface tension. However, recent experimental findings have been contradictory and a definitive explanation has not yet been offered [13]. In order to further investigate this theory, a range of biosurfactant concentrations ranging from above to below CMC were employed during the washing trials. Solutions of the six biosurfactants were prepared using de-ionised water and the CMC was determined using a method outlined previously [17]. A two fold serial dilution was prepared from each surfactant in triplicate, and the surface tension (mN/m) repeatedly measured using a KRUSS tensiometer with a platinum ring.

The solution concentration of all liquid (v/v %) and solid (m/v %) surfactants was calculated as a (w/v %) in solution. Surface tension (mN/m) data was plotted against biosurfactant concentration (log scale) and the CMC of each biosurfactant calculated from the resultant graph (Figure 1). The CMC can be estimated from the graph as the minimum concentration at which the biosurfactant solution reaches its lowest (and then near constant) surface tension measurement.

All six biosurfactant products, rhamnolipids from Biofuture, Cirasolv, Saponin, EC601, EC1800 and Petrosolv (Table 3) were supplied without supplementary information regarding the purity and concentration of the material. The attributes of lowest achievable surface tension and CMC for each of the six commercial biosurfactants were measured and employed as a means to compare the performance of the material. This was the only feasible bench mark for such natural products, which are typically supplied as liquids of unknown dilution. The cost of surfactant in a washing solution at CMC was calculated (for 2007 prices), allowing comparison of the six biosurfactants at optimum dilution as shown in Table 3.

A key finding here is that dilute solutions of microbial secretions (e.g. rhamnolipid) may be competitive with solutions of cheaper plant extracts (e.g. orange oil). The plant based surfactant saponin had the highest cost – however, the material was sourced as a highly pure product from a scientific laboratory supplier. Further suppliers of this material could be sought to determine if a cheaper and bulk source of saponin can be located.



**Figure 1.** Graphical determination of Critical Micelle Concentration by serial dilution and surface tension measurement.

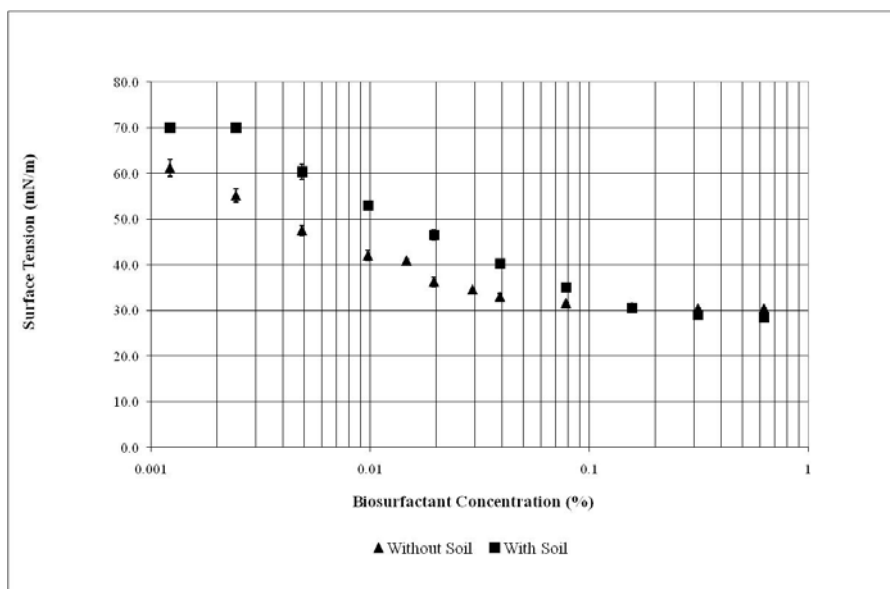
**Table 3.** Summary of biosurfactant characteristics: ST - Surface Tension; CMC Critical Micelle Concentration; Cost of biosurfactant in 1 litre of solution at CMC.

Biosurfactant	Origin	Supplier	ST mN/m	CMC (%)	Cost (£/L)
BioFuture	Bacterial rhamnolipid	BioFuture Ltd Dublin	28	0.08	0.02
Citrasolv	Orange peel	Cleveland Biotech Ltd, Teesside	30	0.9	0.01
EC601	Bacterial rhamnolipid	Ecochem Ltd, Canada	29	0.2	0.23
EC1800	Bacterial consortium	Ecochem Ltd, Canada	28	0.04	0.01
Petrosolv	Bacterial unknown	Enzyme Technologies Inc, USA	34	0.2	0.01
Saponin	Plant bark	Sigma UK	45	0.1	0.50

### 2.3.2. Impact of soil sorption on biosurfactant critical micelle concentration

The adsorption of biosurfactant molecules onto the surface of the washed soil will remove surfactant particles from solution and will therefore lead to a lower effective concentration. The effect of soil adsorption was investigated by adapting a previously published method [13]. A 2-fold serial dilution was prepared from the BioFuture biosurfactant sample. Each biosurfactant dilution was mixed with garden soil in a 1:6





**Figure 2.** Plot of surface tension (mN/m) plotted against concentration both with (■) and without (▲) mixing with soil.

ratio of soil to solution and shaken for 24 hours at 150 rpm. The soil was removed by centrifugation and a CMC plot prepared for the separated biosurfactant solutions. The CMC plot for BioFuture biosurfactant with and without the addition of soil is shown in Figure 2. The difference between the CMC of the biosurfactant solution with and without soil mixing indicated that approximately 60 % of the biosurfactant was adsorbed onto a clay soil. Such loss of surfactant from solution needs to be considered when planning washing experiments and could affect the cost of the biosurfactant washing process if this observation applied to all wastes and soils.

### 2.3.3. Oil solubilisation by biosurfactant

The partitioning of oil into micelles was proposed [18] as one of the potential mechanisms by which a surfactant removes oil from contaminated soil particles and is referred to as solubilisation. An alternative mechanism has also been proposed, often referred to as soil roll up or mobilisation [18,19]. The occurrence of both mechanisms during soil washing was tested using a method described previously [15]. Three concentrations of the rhamnolipid biosurfactants (BioFuture and Citrasolv) were prepared at approximately 0.1, 1 and 10 times their CMC. The solutions were mixed at 200 rpm for 1 hour with a 1 % addition of crude oil, followed by settling for 1 hour in a separating funnel. A portion of each biosurfactant solution was removed and tested for TPH content by a commercial laboratory (Severn Trent Laboratories). Chemical analysis of the liquids showed that no TPH had been extracted from the biosurfactant solution, indicating that the biosurfactants tested were not forming a stable emulsion or solubilising the crude oil for any length of time. This appears to support the previous assertions [17] that mobilisation is the more likely mechanism for pollutant removal and that micelle formation (and CMC concentration) is not critical for removing oil particles from the soil

surface. However, later experimental data indicates that micelles could play an important role in aiding the separation of finer soil particles from the soil matrix by solubilisation, and this process can enhance the removal of contamination.

## **2.4. Optimisation of biosurfactant washing**

### **2.4.1. Soil washing method**

The key factors predicted to affect soil washing were: biosurfactant concentration, wash time, waste: liquid ratio, agitation, and solution temperature. The following method was designed to allow testing of these parameters, using a laboratory scale washing process derived from British Standard BS 12457/3 [16].:

100g of waste was transferred to a reinforced glass 2 litre bottle with a Teflon lined lid; a pre-determined concentration and volume of biosurfactant solution was added to the soils and mixed thoroughly; bottles were placed on rollers and mixed at 9 rpm for a pre-determined time; bottles were removed and settled for 1 hour, after which solution was removed by peristaltic pump and up to 250 ml retained at 4°C for further analysis; wastes were rinsed with addition of 100 ml de-ionised water and horizontal rotary mixing at 100 rpm for 1 minute, followed by settling for 30 minutes; rinse water was removed by pipetting and 30 ml was stored at 4°C for further analysis; wastes were transferred to glass evaporating dishes and dried in a fume extraction hood at room temperature for 24 – 48 hours; dried wastes were weighed and transferred to sealed bags and stored at 4°C for further analysis. Any modifications made to this method during individual experiments are described in that section.

### **2.4.2. Initial soil washing study**

Initial testing of biosurfactant soil washing used two of the target wastes (oily mill scale & gas works soil) and three biosurfactants (BioFuture, Citrasolv & Saponin) at three concentrations (0.1, 1 and 10 factors of CMC). Garden soil and water were included as controls. TPH and TOC were determined on the washed soils by a commercial laboratory (Severn Trent Laboratories). The analytical results indicated that TPH concentration had apparently increased in many of the washed soils (Table 4). The data were therefore normalized to the apparent concentration reported in the water-washed control (Table 5) to eliminate this effect (discussed below).

The TOC content of many of the wastes was reduced by the washing process, the extent of which can be seen in Table 6. However, the addition of biosurfactants did not appear to provide any benefit over washing with water alone, except in a few cases. Where the TOC level increased, this could indicate adsorption of the biosurfactant, an organic molecule, onto the soil surfaces, or transfer during washing to the finer grain size fraction (<2mm) in the analytical sample. It was clear that some reduction of organic contaminant occurred during the washing process, albeit often a modest one. However, the identity and quantity of the removed contaminants was not clear from the data obtained, possibly due to sample heterogeneity.

Linear regression analysis was performed on the TPH and TOC data to determine any patterns. No relationship was observed between the TPH and TOC concentrations. Additional biosurfactant was not found to have a significant effect on the physico-chemical removal of oil during the washing process.

The counter-intuitive results of increased TPH in washed samples were presumed at this stage to indicate that the washing process had substantially increased the chemical

**Table 4.** Final TPH concentration of wastes: oily mill scale, gas works soil and garden soil, calculated as a percentage (%) of their initial (pre-wash) concentration. Data in bold show apparent increase, data in italic show decrease.

	Concentration (CMC x)	SAMPLES		
		Oily Mill Scale	Gas Works Soil	Garden Soil
<b>BioFuture</b>	<b>0.01</b>	<b>116</b>	<b>157</b>	<b>102</b>
	<b>0.1</b>	<b>106</b>	<b>179</b>	79
	<b>1</b>	<b>106</b>	<b>164</b>	<b>250</b>
<b>Citrasolv</b>	<b>0.1</b>	84	<b>150</b>	<b>102</b>
	<b>1</b>	<b>742</b>	<b>207</b>	<b>212</b>
	<b>10</b>	<b>126</b>	<b>464</b>	<b>137</b>
<b>Saponin</b>	<b>0.01</b>	<b>110</b>	<b>200</b>	98
	<b>0.1</b>	<b>148</b>	<b>164</b>	69
	<b>1</b>	<b>139</b>	<b>157</b>	100
<b>Water</b>	n/a	<b>129</b>	<b>164</b>	73

**Table 5.** Results from Table 4 normalised to the water-washed control. Data in bold show apparent increase relative to water washing, data in italic show decrease.

	Concentration (CMC x)	SAMPLES		
		Oily Mill Scale	Gas Works Soil	Garden Soil
<b>BioFuture</b>	0.01	90	96	<b>139</b>
	0.1	83	<b>109</b>	<b>108</b>
	1	83	100	<b>342</b>
<b>Citrasolv</b>	0.1	65	91	<b>139</b>
	1	<b>575</b>	<b>126</b>	<b>289</b>
	10	98	<b>283</b>	<b>187</b>
<b>Saponin</b>	0.01	85	<b>122</b>	<b>134</b>
	0.1	<b>115</b>	100	95
	1	<b>108</b>	96	<b>137</b>
<b>Water</b>	n/a	100	100	100

availability of the contaminants to the organic solvent used in the analytical extraction. The un-normalised TPH data showed that the addition of biosurfactant increased this effect in nine washes out of ten for the control soil. However, the effect upon oily mill scale and gas works soil is ambiguous, with some washed soils having elevated levels of hydrocarbons, and others lowered. Using this data set alone it is not possible to identify a pattern.

#### 2.4.3. Factors affecting soil washing

A thorough investigation of the soil washing process was undertaken to allow further examination of the earlier observations and factors affecting oil removal. The experiments

**Table 6.** Final TOC concentration of wastes: oily mill scale, gas works soil and garden soil, calculated as a percentage (%) of their initial TOC. Data in italic show decrease relative to water washing, data in bold show apparent increase relative to water washing, data in bold italic show apparent increase relative to unwashed sample.

	Concentration (CMC x)	SAMPLES		
		Oily Mill Scale	Gas Works Soil	Garden Soil
<b>BioFuture</b>	0.1	92	<b>84</b>	<b>100</b>
	1	92	<b>84</b>	<b>97</b>
	10	<b>93</b>	<b>88</b>	<b>104</b>
<b>Citrasolv</b>	0.1	<b>97</b>	<b>91</b>	<b>111</b>
	1	<b>102</b>	<b>109</b>	<b>110</b>
	10	<b>113</b>	<i>63</i>	<b>126</b>
<b>Saponin</b>	0.1	<b>98</b>	<b>84</b>	<b>99</b>
	1	<b>100</b>	<i>67</i>	<i>83</i>
	10	<i>79</i>	<b>81</b>	<b>100</b>
<b>Water</b>	n/a	92	<i>77</i>	<i>89</i>

**Table 7.** Design outline of soil washing experiments showing factors and levels tested.

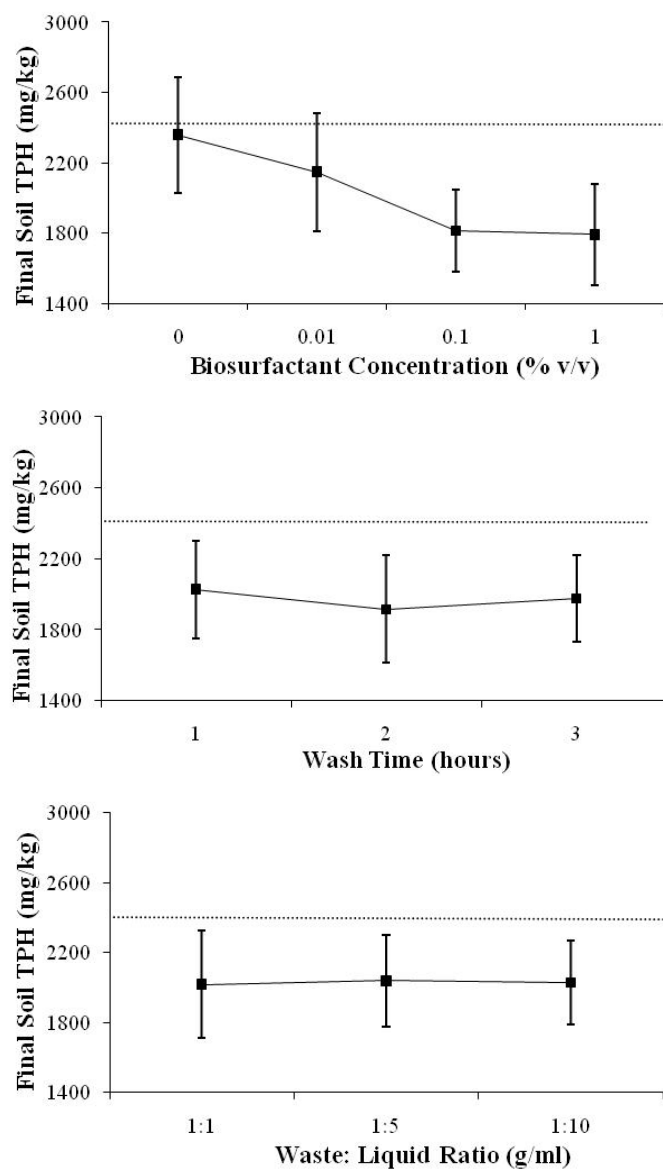
Factors Tested	Levels Tested		
	1	2	3
Biosurfactant Concentration (%)	0.01	0.1	1
Waste:Liquid Ratio (g/ml)	1:1	1:5	1:10
Washing Time (hours)	1	2	3

were designed using a three factor, three level (3 x 3), factorial design to allow thorough statistical analysis of the results. The conditions tested are outlined above (Table 7). Each wash was performed in triplicate.

Throughout the experiment the waste tested was gas works soil, the biosurfactant was BioFuture rhamnolipid, and the control was water washing. Due to the anticipated heterogeneous nature of the organic contaminants in the gas works soil, a pre-treatment waste preparation method was undertaken as follows:

- Soil was homogenized using a cement mixer and air dried for 24 hours in a fume hood
- Dried soil was sieved to <4mm and stored in an air tight polypropylene container prior to experimentation and analysis

Overall, 108 washed soils were analysed for TPH content by a commercial laboratory (Severn Trent Laboratories). This number includes the 81 samples washed with biosurfactant and 27 samples washed with water as a control. In addition, the unwashed soil, laboratory water supply (used to dilute biosurfactant), final wash water, and biosurfactant wash solution (0.01, 0.1 & 1 %) were analysed in triplicate for TPH content.



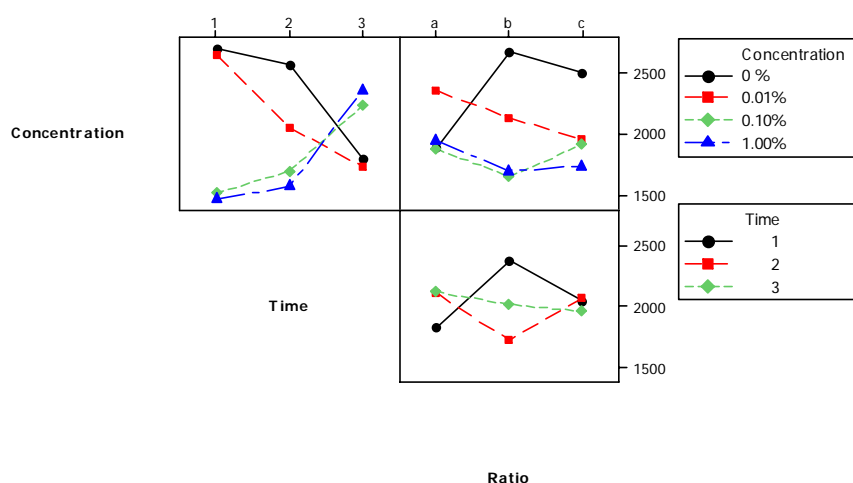
**Figure 3.** Effect of factors: biosurfactant concentration (% v/v), wash time (hours) and waste:liquid ratio (g/ml) on soil TPH concentration after washing. The points represent an average of 24, 36 and 36 determinations respectively. Error bars represent two standard errors of the mean. Horizontal dashed line represents the concentration of TPH in the unwashed soil (2400 mg/kg).

The results were analysed in Minitab (version 14) using a multi-factor ANOVA, to determine the effect of biosurfactant concentration, waste: liquid ratio and washing time on washed soil TPH concentration. The results indicated that biosurfactant concentration had a significant effect on final soil TPH ( $\alpha = 0.05$  level), with a higher biosurfactant

concentration leading to a lower final TPH concentration. These findings contrast with the initial results for the unsieved, undried and unhomogenized portion of the same soil (gas works soil, Table 4), particularly for the water wash. This indicates that biosurfactant micelles may play a role in removal of TPH from the gas works soil tested. Whether this is indeed due to the direct transfer of hydrocarbons from the soil surface to the micelle core, as in the postulated mechanism of solubilisation, cannot be determined from this data alone. Further results indicated that the waste:liquid ratio and washing time did not have a significant effect on TPH removal. The effect graphs for the three factors are shown in Figure 3.

The liquid wash water was tested for TPH content to determine if any transfer of TPH from solid to liquid had taken place. The results were inconclusive. In particular, it was found that when the whole washwater was mixed together, the TPH content was 1600 mg/litre. On initial inspection this was thought to potentially be due to the presence of transferred TPH from the soil during washing. However, the clean biosurfactant solutions (0.01, 0.1, 1 % v/v) and water were also tested and the TPH content was found to be 2400, 100, 270 and 100 mg/litre respectively. From these results it can be seen that the biosurfactant solution at a concentration below its CMC (0.01 %) gave a TPH value of 2400 mg/litre when subjected to solvent extraction and gas chromatography. This indicates that potentially there is some interference of the biosurfactant monomer with the chemical analysis of the liquid wastes. Further studies will need to be conducted to determine the extent and source of this interference.

An interaction plot was prepared, which allows any relationship between the factors tested to be identified (Figure 4.). The interaction between biosurfactant concentration and wash time was found to be significant at  $\alpha = 0.05$  level. It can be seen that in washed soil TPH is lower after a wash time of 1 hour, when washed with a biosurfactant solution above CMC (i.e. 0.1 and 1 %), whereas a concentration of biosurfactant below CMC (0.01 % and water) leaves a significantly higher concentration in the soil. This interaction is shown to reduce in significance as the wash time increases to 2 and 3 hours. There were no other significant interactions observed between the factors.



**Figure 4.** Interaction plot showing the relationship between biosurfactant concentration (0, 0.01 %, 0.1 % and 1 %), wash time (1, 2 and 3 hours) and waste:liquid ratio (a [1:1], b [1:5] and c [1:10]).

#### 4. Summary of experimental results

The main results from the experimental work are as follows:

- Washing did not conclusively appear to produce the anticipated movement of TPH into the liquid phase or allow recovery as NAPLS – A key finding for the study.
- Addition of wash water above a waste to liquid ratio of 1:1 (g:ml), had no significant effect on TPH removal from the soil. This suggests that the TPH is not being removed into the liquid phase, as additional solution does not lead to enhanced removal.
- Increasing the time of soil washing to above 1 hour did not have a significant effect on TPH removal from soil. This data suggests that all transfer of TPH was achieved with the first 60 minutes of treatment.
- Addition of biosurfactant to the wash water during washing was found to have a significant effect on TPH removal from the soil matrix.

Several complicating factors were also surmised:

At low agitation rates and surfactant concentrations mixing of the waste is inadequate; Decanting wash-water can transfer suspended fines with adsorbed TPH unless allowed to settle;

Below CMC biosurfactant present as monomers (i.e. not micelles) is likely to bind to fines, reducing potential adsorption of TPH;

Above CMC the dispersion of suspended fines is enhanced;

The following method statement was devised for soil washing trials with biosurfactant following our protocols:

- The concentration of a rhamnolipid biosurfactant (e.g. BioFuture) should be above its CMC, which will lead to enhanced transfer of TPH to fine particles and enhanced recovery of these fines after settling from the wash water.
- The agitation speed should be sufficient to allow thorough mixing of the liquid and solid phases (further trials to be conducted).
- The ratio of solid waste to liquid should be approximately 1 to 2, but an increase in liquid has no effect.
- A washing time of 1 hour is sufficient for batch washing to be completed.

#### 5. Conclusions and implications

1. Conventional soil-washing involves the physical separation of a fraction of the soil which contains a disproportionately high percentage of the contamination, usually the finer particles. It is a cost effective remediation process for sandy soils where, for example, the bulk of the heavy metals are associated with a 10% clay fraction which can be disposed of to landfill. Our review of current remediation practice indicated that organic contaminants can also be removed - by virtue of the higher surface area of the fine fraction, onto which they are adsorbed. In our laboratory trials the extent of the removal was variable, waste-specific and dependent on a number of factors.
2. Factors affecting the potential use of commercially available biosurfactants in soil-washing include:
  - the achievable reduction in surface tension (ST)
  - the CMC - the concentration of surfactant in solution at which the surfactant molecules aggregate together to form spherical structures (micelles), which provides a means of comparing the efficacy of different products, particularly rhamnolipids

- biosurfactant depletion by adsorption on the surface of clay particles in soils
  - The combination of variable CMC, ST and cost as supplied combine to determine the economics of a particular washing solution. If it is assumed that CMC provides a valid basis for comparison of use in soil-washing, then more dilute solutions of microbial secretions (e.g. rhamnolipid) may still be economically competitive with stronger solutions of cheaper plant extracts (e.g. orange oil).
3. Washing studies on TPH- and PAH-contaminated soil indicate that enhanced transfer to finer (clay) size fractions occurs with rhamnolipid biosurfactant concentrations above CMC, which infers that removal of organic contaminants from sand and coarser fractions is enhanced. This indicates that addition of biosurfactant could potentially improve the performance of conventional soil-washing by enhancing the liberation of fines, leading to lower residual contamination in reusable grain size fractions. Initial trials to introduce a clay fraction to granular oily wastes as a carrier were inconclusive.
  4. Transfer of TPH to biosurfactant washing solution through solution, solubilisation in micelles, or formation of floating product was minimal in the weathered wastes studied, which contradicted earlier studies of spiked samples in the literature. This indicates that soil-washing is unlikely to generate an additional liquid waste requiring (or amenable to) bioreactor treatment. This also means that contaminants cannot be adequately removed through movement to wash water to avoid subsequent disposal of the finer fraction generated by soil-washing.

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